Journal of Basic and Clinical Pharmacy

www.jbclinpharm.com

ANTI-INFLAMMATORY ACTIVITY OF *MIRABILIS JALAPA* LINN. LEAVES

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ABSTRACT: *Mirabilis Jalapa Linn. is a widely used traditional medicine in many parts of the world for the treatment of various diseases viz. virus inhibitory activity, anti tumour activity. It is claimed in traditional medicine that the leaves of the plant are used in the treatment of inflammation. In the present study, the total alcoholic extract and successive petroleum ether fractions of leaves of Mirabilis Jalapa Linn were screened for its anti-inflammatory activity using carageenan induced rat paw edema and cotton pellet induced granuloma models. The total alcoholic extract at the dose of 300 mg/kg p.o and successive petroleum ether fraction at the dose of 200 mg/kg exhibited significant anti-inflammatory activity in carrageenan induced paw edema model ($p<0.01$). In cotton pellet granuloma model, the total alcoholic extract at the dose of 300 mg/kg and successive petroleum ether fraction at the dose of 200 mg/kg inhibited granuloma formation significantly ($p<0.05$) indicating that both test samples inhibit the increase in number of fibroblasts and synthesis of collagen and mucopolysaccharides during granuloma tissue formation during the chronic inflammation. These experimental results have established a pharmacological evidence for the folklore claim of the drug to be used as an anti inflammatory agent.*

KEYWORDS:
Anti inflammatory, *Mirabilis Jalapa*, Traditional Medicine.

received on 19-03-2010

modefied on 12-04-2010

accepted on 30-04-2010

available online 15-05-2010

www.jbclinpharm.com

INTRODUCTION

Inflammatory diseases are becoming common in aging society throughout the world. Recent studies indicate that the mediators and cellular effectors of inflammation are important constituents of the local environment of tumors.[1] Natural products in general and medicinal plants in particular, are believed to be an important source of new chemical substances with potential therapeutic efficacy.[2] Mirabilis Jalapa Linn. is a widely used traditional medicine in many parts of the world for the treatment of various diseases viz. virus inhibitory activity, anti tumour activity.[3] *Mirabilis Jalapa* Linn (Nyctaginaceae) is a perennial herb and is known as "Gulambasa" in ayurveda. It produces beautiful flowers that usually open around 4o'clock in the afternoon-hence its common name, four *o'clocks*.[4] The presence of oxymethyl anthraquinone, trigonelline, arabinose, galactose, beta-sitosterol in leaves has been reported.[5] It is used in the traditional system of medicine in the treatment of piles, abscess, boils and ulcers.[6] The leaves and stems are used as a diuretic and tonic. Juice of leaves is applied to wounds and bruises for allaying itching in diseases like urticaria in traditional systems of medicine.[7] The phytochemical and pharmacognostical studies of the leaves of Mirabilis Jalapa have been recently reported by our group.[8] It is claimed in traditional medicine that the leaves of the plant are used in the treatment of inflammation.[9] However there is no scientific proof justifying the traditional use of leaves in the treatment of inflammation. Hence, the present work was undertaken to evaluate the anti inflammatory activity of *Mirabilis Jalapa* Linn leaves.

MATERIALS AND METHODS

Plant material

The leaves of *Mirabilis Jalapa* Linn. were collected from the local areas of Hubli, Karnataka, and

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authenticated by Dr. B.D. Huddar, Head, Department of Botany, H.S.K. Science Institute, Hubli, India.

Extraction of crude drug

The extraction of crude drug was carried out according to the method reported elsewhere.[10] In brief, the coarsely powdered leaves were extracted with 95% alcohol (in 4 batches of 250g each) using soxhlet extraction apparatus. The solvent was completely removed under reduced pressure to yield total alcoholic extract (14%). 40g of total alcoholic extract were dispersed in double distilled water (200 mL), successively fractionated thrice with 200 mL portions of pet ether (40-60°C) using separating funnel. The pet ether fractions were separated, concentrated under reduced pressure to yield successive pet ether fractions (8.5%). The qualitative chemical investigation of the total alcoholic extract and successive pet ether fractions were carried out to check the presence of various phytoconstituents. Our earlier reported studies have revealed the presences of steroids, alkaloid, flavonoids, carbohydrates, glycosides, and proteins in the total alcoholic extract and presence of steroids in successive pet ether fractions.

Animals

Albino rats of Wistar strains of either sex between 150-200g were purchased from University of Agricultural studies, Dharwad. Gender of the rats does not make a difference for inflammatory activity as reported in many earlier studies.[11,12] The animals were kept on diet and allowed food and water *ad libitum*. They were housed in polypropylene cages maintained under standard conditions.

Preparation of sample

The total alcoholic extract and successive pet ether fractions were suspended in Tween 80 (0.5%) in normal saline (vehicle) and were used for anti inflammatory activity studies. Tween 80 (0.5%) was used as it is the commonly used suspending agent in earlier reported studies.[13]

Acute toxicity study:

Acute toxicity studies were carried out as per fixed dose OECD guidelines No: 420 using albino mice.[14] In brief, albino mice of either sex weighing between 20-30g were used for acute toxicity study. The animals were fasted over night prior to the experimental procedure. The animals were kept for fasting overnight providing only water, after which the extracts were administered orally at the dose level of 5 mg/kg body weight and observed for 7 days. If mortality was observed in 2 out of 3 animals, then the dose administered was assigned as toxic dose. If mortality was observed in 1 animal, then the same dose was repeated again to confirm the toxic dose.If mortality was not observed, the procedure was repeated for further higher doses such as 50, 300 and 2000 mg/kg body weight.

Evaluation of anti inflammatory activity:

Anti inflammatory activity were evaluated using acute and chronic inflammatory models. Acute anti-inflammatory activity was evaluated by carrageenan – induced rat paw edema method and the chronic inflammatory activity was evaluated by cotton pellet granuloma model. The ethical clearance was obtained by the Institutional Animal Ethics Committee (Registration No: 126/1999/CPCSEA) before carrying out the experiment.

Carrageenan induced paw edema model:

The acute anti-inflammatory activity was evaluated by carrageenan – induced rat paw edemas as earlier described by Turner et al.[15] In brief, Wistar albino rats were divided into 4 groups (n=6). Acute inflammation was produced by injecting 0.1 mL of 1% carrageenin into sub- plantar surface of rat hind paw. The control group received tween 80 (0.5%) 0.1mL. The test group 1 and 2 received 300 mg/kg total alcoholic extract, 200 mg/kg successive pet ether fraction respectively by oral route. The standard group received comparator drug diclofenac 40 mg/kg by oral route. All the suspensions were administered 30 minutes before carrageenan injection (0.1mL of 1%). The paw volume, up to the tibiotarsal articulation, was measured using a plethysmometer at 0, 1, 2, 3, 4, 5, & 6 hrs.

Cotton pellet granuloma model:

The chronic inflammatory models were evaluated by cotton pellet granuloma model described elsewhere.[16] Wistar albino rats were divided into four group (n=6). The animals were anaesthetized with

ether; the back skin was shaved and disinfected with 70% ethanol. An incision was made in the lumbar region. Subcutaneous funnels were formed by using blunted forceps and subsequently sterilized cotton pellets weighing 20 ± 1mg were implanted on either sides of the scapular region of each rat. Group I served as control and received the vehicle. The total alcoholic extract at concentration of 300 mg/kg and successive pet ether fraction at 200 mg/kg was administered orally to group II and group III animals for 7 days. Group IV animals received diclofenac at a dose of 40 mg/kg p.o for the same period. On the eighth day, the animals were sacrificed and the pellets together with the granuloma tissues were carefully removed, dried in an oven at 60°C, weighed and compared with control (Increment in the dry weight of the pellets is taken as a measure for granuloma formation).

STATISTICAL ANALYSIS

The experimental data were expressed as the mean ± SE. The standard error of the mean (SEM) is the standard deviation of the sample mean estimate of a population mean. SEM is estimated by the sample estimate of the population standard deviation (sample standard deviation) divided by the square root of the sample size. Statistical analysis was carried out using one- way analysis of variance followed by Dunnett's Multiple Comparison Test and p values implied significance($p<0.01$).[17]

RESULTS AND DISCUSSION

Acute toxicity test

LD 50 were found to be 3000 mg/kg body weight for total alcoholic extract and 2000 mg/kg body weight for pet ether fractions. According to OECD guidelines for acute oral toxicity, an LD50 dose of 2000 mg/kg-[1] and above is characterized as unclassified and hence the drug is found to be safe. As a result, the dose were fixed at 300 mg/kg body weight, 200 mg/kg body weight for total alcoholic extract and successive pet ether fraction respectively as it was 1/10 of the LD 50 dose. Preliminary phytochemical screening reported earlier revealed the presences of steroids, alkaloid, flavonoid, carbohydrates, glycosides, and proteins in total alcoholic extracts and presence of steroids in successive petroleum ether fractions.[8]

The total alcoholic extract at the dose of 300 mg/kg p.o and successive pet ether fraction at the dose of 200 mg/kg exhibited significant anti-inflammatory activity in carrageenan induced paw edema model ($p<0.01$) (Table-1). It is well known that carrageenan induced paw edema model is commonly used as an experimental model for evaluating anti inflammatory activity of natural products[18] and is believed to be bi phasic, of which the first phase is mediated by the release of histamines and 5 hydroxy tryptamin in the early stage followed by kinin release and then prostaglandin in the later stage. In cotton pellet granuloma model, the total alcoholic extract at the dose of 300 mg/kg and successive pet ether fraction at the dose of 200 mg/kg inhibited granuloma formation significantly ($p< 0.05$) (Table-2). This indicates that both test samples inhibits the increase in the number of fibroblasts, synthesis of collagen and mucopolysaccharides during granuloma tissue formation in the chronic inflammation stage.[19] In this study, an attempt has been made to evaluate the anti inflammatory activity of *Mirabilis*

Table 1: Anti-inflammatory activity of total alcoholic extract & successive pet. Ether fractions of *Mirabilis Jalapa* linn. (Leaves) on carrageen induced paw edema model

Group (n=6)	Dose (mg/Kg)	Paw volume (Mean ± S.E)	Paw edema vol in ml (Mean ± S.E)			
			1 Hr	2 Hr	3 Hr	6 Hr
Control	0.5% Tween 80	0.23 ± 0.01	0.43 ± 0.06	0.53 ± 0.03	0.64 ± 0.02	0.67 ± 0.01
Diclofenac	40	0.15 ± 0.03	0.23 ±0.04	0.29 ± 0.01	0.31 ± 0.12	0.27 ± 0.08*
Total Alcholic Extract	300	0.21 ± 0.01	0.28 ± 0.01	0.31 ± 0.01	0.37 ± 0.02	0.26 ± 0.01*
Succ.Pet ether fraction	200	0.18 ± 0.08	0.23 ± 0.08	0.29 ± 0.06	0.32 ± 0.05	0.23 ± 0.04

*Significant at $p<0.01$, p-values calculated by comparing with control by ANOVA followed by Dunnet test.

Table 2: Anti-inflammatory activity of total alcoholic extract and successive pet ether fractions of *Mirabilis Jalapa* linn (leaves) on cotton pellet granuloma model

Group	Dose (mg/kg)	Dry weight of cotton pellet in mg (Mean ± S.E.M)	% inhibition
Control	-	69.85 ± 0.0631	-------
Diclofenac	40	33.77 ± 0.0637*	51.65%
Total Alcoholic extract	300	43.89 ± 0.063*	37.16%
Succ.Pet ether	200	39.87 ± 0.063*	42.92%

*Significant at p<0.05 compared to control group.

Jalapa Linn. This study revealed that total alcoholic extract and successive petroleum ether fractions possesses good anti inflammatory property which may be attributed to the individual or combined action s of phytoconstituents like alkaloids and steroids present in it. These experimental results have established a pharmacological evidence for the folklore claim of the drug to be used as an anti inflammatory agent. Further study regarding the isolation and characterization of bio active principles are currently under progress.

ACKNOWLEDGEMENTS

The authors are thankful to the management KLE society, Belgaum and Principal, KLES College of Pharmacy, Hubli for providing the necessary facilities to carry out this study.

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